

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Lack of effect of bicarbonate on the survival of *Treponema pallidum* (Nichols) in vitro

Sir,
No published studies have reported the effect of bicarbonate (HCO_3^-) on the survival of *Treponema pallidum* in vitro.

Carbon dioxide (CO_2) has been shown to be beneficial to *Neisseria gonorrhoeae*, *N meningitidis*, some staphylococci,¹ and other bacteria.^{2,3} CO_2 serves as a carboxylating agent⁴ and as an essential precursor for the synthesis of certain amino acids.⁵ It is also required for fatty acid synthesis,^{1,6} biosynthesis of purine ribonucleotides, which are precursors of DNA and RNA and are an integral part of many coenzymes.⁶

Different concentrations of sodium bicarbonate (NaHCO_3) were used in both a cell-free and cell-culture system to determine whether bicarbonate had any beneficial effect on the survival of *T pallidum* in vitro.

The medium in the cell-free system⁷ was slightly modified by replacing the sodium thioglycollate with 2 mmol/l dithiothreitol (DTT). In the cell-culture system, Eagle's minimal essential medium with 10% heat-inactivated fetal calf serum, 0.5 mmol/l DTT, and 20 mmol/l N-2-hydroxyethyl-piperazine-N-2-ethane-sulphonic-acid (HEPES) was used.

T pallidum was harvested from infected rabbit testes.⁷ Anaerobic incubation has been described.⁷ For microaerophilic incubation, tubes with cottonwool plugs were equilibrated with 3% O_2 ⁷ for two days before inoculation with treponemes. Treponemes were then added to an approximate final density of $5 \times 10^6/\text{ml}$, followed by HCO_3^- to give a final concentration of from 10^{-5} to 10^{-2} mol/l. The cottonwool plugs were immediately changed to rubber stoppers to prevent escape of CO_2 . The pH remained at 7.3 ± 0.2 in the cell-free system. The incubation temperature was 34°C .

The isolation and subculture of the rabbit testicular cell lines have been described.⁸

Viability of *T pallidum* in vitro was determined by the percentage motility under darkfield microscopy and their virulence in rabbits.⁷ In the cell-culture

system, attached treponemes were separated from tissue cells⁸ before virulence was tested.

Ten experiments were performed in a cell-free medium under anaerobic conditions; a representative experiment is shown in the figure. There was no enhancement by HCO_3^- (10^{-2} to 10^{-5} mol/l) on the retention of motility of *T pallidum* (figure; a); the percentage motility in the presence of 10^{-3} and 10^{-2} mol/l HCO_3^- was consistently lower than the control. Similarly, HCO_3^- (10^{-2} to 10^{-5} mol/l) had no beneficial effect on the retention of virulence of *T pallidum* in vitro (figure; b).

Bicarbonate (10^{-5} to 10^{-2} mol/l) showed no enhancement of treponemal survival in two experiments under microaerophilic conditions with 3% O_2 . The percentage motility (figure; c) and latent periods (figure; d) were virtually the same at all concentrations tested and were not appreciably different from the control tubes lacking bicarbonate.

In three experiments in cell-cultures, the pH of the medium dropped to 6.8 at day 4 in the control tubes while remaining at 7.2 in the presence of $\text{HCO}_3^-/\text{CO}_2$. When the pH of the medium was regularly adjusted, however, HCO_3^- (10^{-4} to 10^{-2} mol/l) had no enhancing effect on the retention of treponemal motility (figure; e) or virulence (figure; f) in the presence of tissue culture cells. Thus, in the media tested, $\text{HCO}_3^-/\text{CO}_2$ may serve mainly as a buffering system rather than as a reactant in any essential metabolic process.

Possible reasons why $\text{HCO}_3^-/\text{CO}_2$ had no enhancing effect on treponemal survival in vitro may be: (a) CO_2 requirements were satisfied by its endogenous metabolism, such as degradation of pyruvate and glucose.^{9,10} (b) *T pallidum* under conditions which do not allow multiplication may have no capacity to use CO_2 even if it normally utilises it. (c) Even if *T pallidum* had the capacity to incorporate CO_2 , it may be unnecessary. Since the medium was extremely rich, the presence of these nutrients may have shut down certain biosynthetic pathways. The incorporation of $\text{NaH}^{14}\text{CO}_3$ was insignificant when compared to total glucose incorporated.¹⁰ (d) CO_2 would not be used for fatty acid synthesis since *T pallidum*, like other treponemes, cannot synthesise fatty acids.¹¹ (e) $\text{HCO}_3^-/\text{CO}_2$ may serve as a buffer in a

closed system, but with HEPES (20 mmol/l) serving this purpose $\text{HCO}_3^-/\text{CO}_2$ was unnecessary in both the cell-free and cell-culture systems.

We gratefully acknowledge the excellent technical assistance provided by Mrs L Drummond and thank Professor S Faine and Dr Ben Adler for constructive critical evaluation. This work was supported by grants from Australian National Health and Medical Research Council (NR & MRC); the Utah Foundation, the Heiser Program, and Monash University.

Yours faithfully,

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